

The University of Chicago

THE ORIGIN OF CASTES IN ANTS
WITH SPECIAL REFERENCE TO
PHEIDOLE MORRISI FOREL

A PART OF A DISSERTATION SUBMITTED TO
THE FACULTY OF THE DIVISION OF THE
BIOLOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

1941

By

ROBERT E. GREGG

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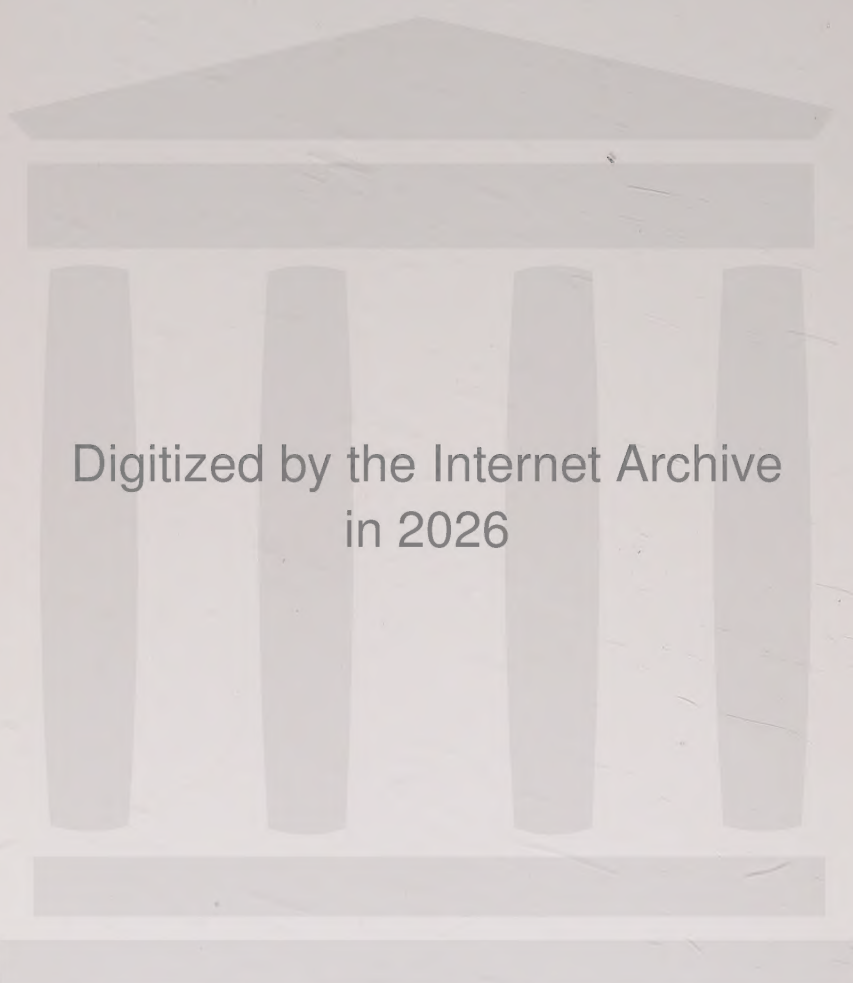
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THE ORIGIN OF CASTES IN ANTS WITH SPECIAL REFERENCE TO *PHEIDOLE MORRISI* FOREL¹

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INTRODUCTION

The controversy in respect of the determination of castes in the social insects has had a long history. The four principal groups of these insects have each received varying amounts of attention, but until recently only the polymorphism of bees and wasps yielded to an explanation which may be supported by experimental information. It appears that the caste origin of termites may now be partly understood (Castle, '34a, '34b), but the problem in ants has remained obscure despite the latest interpretations of Wheeler ('37).

HISTORICAL

Polymorphism embraces such phenomena as alternation of generations or metagenesis, sexual dimorphism, cyclo-morphism, metamorphosis and the social polymorphism of ants, bees, wasps and termites. Ford ('40) allows the term an even wider application by including the multiple forms or phases in which a species may exist. However, Wheeler ('10, p. 86) restricted its usage to the polymorphism of social insects, and for clarity this meaning is adopted in the present report.

Castes in social insects are commonly associated with physiological division of labor, and the behavior and morphological characteristics of the individual are also involved. Although both phylogenetic and ontogenetic sequences in the

origin of castes are described there still remains the elucidation of the precise physical and chemical processes that are responsible for their development. Such processes have been investigated in the honeybee and termites with encouraging results, but many refinements in the analyses are needed before an adequate knowledge of the development of castes can be expected. Whether caste differences are to be attributed alone to germinal causes contained within the egg, or are the result of various extrinsic or ecological influences acting on the organism from without is still a controversial question. Among students of the social insects, the problem is referred to as blastogenesis (genetic origin) versus trophogenesis (nutritional origin). Much space is devoted to a discussion of the subject by Wheeler ('10, chaps. 6, 7; '28, chaps. 7, 8), and the bulk of evidence in the literature seems to point toward some sort of environmental control of caste origin.

Santschi ('20) maintained that the solution of the question depended upon the discovery of a caste mosaic in which parts of two subcastes of the female sex would be combined in one individual. Santschi's prediction seemed to be fulfilled when Tulloch ('32) reported a specimen of this nature belonging to the primitive Australian genus, *Myrmecia*. It was termed a "gynergate," descriptive of its union of queen and worker characters. This case has been greatly augmented by Wheeler's treatment ('37) of forty-six gynergates in the fungus-growing ant, *Acromyrmex octospinosus*. Wheeler thought these data were convincing evidence of hereditary differences in the eggs. The anomalies were reinterpreted independently by Whiting ('38) and Gregg ('38) on the basis of physio-

¹ The writer deeply appreciates the helpful guidance of Dr. A. E. Emerson under whose direction this study was made. Many thanks are extended to Dr. W. C. Allee for important criticisms and advice, to Drs. Sewall Wright and Thomas Park for aid with the statistical analysis, and to Dr. M. R. Smith for confirmation of specific determinations. Dr. S. F. Light has offered valued comments concerning termite castes.

logical thresholds, and were considered of trophogenic origin.

Ezhikov ('34) refers to some experiments on *Camponotus* in which larvae were subjected to hunger without producing intergrades between the queen and worker castes. No data are cited, however, and one cannot evaluate his results.

Spencer ('94) quoted the investigations of J. H. Hart of Trinidad, and was in full accord with the view that the social castes are wholly determined by the treatment of the larvae. In one of Mr. Hart's colonies of fungus-growing ants (species not stated) the queen was removed and in her place several new queens developed. The majors, media and minors developed as in a normal colony. In another nest from which the queen was not removed, only workers were produced. The rearing of workers alone in this second nest continued for eighty days, and no males or winged females appeared in that time. These results are reminiscent of those of Grassi and Sandias ('93-'94) on termites. Unfortunately, Hart's data are few and are not quantitatively documented.

Somewhat similar experiments were performed by Emery ('21) but without completely valid controls. In a colony of *Aphaenogaster testaceopilosa spinosa* he was able to rear only workers for two years. Two months after the queens had been removed, three of the larvae became very large and one of these transformed into a winged female. Using *Pheidole pallidula*, he repeated the experiment, removing the queens and observing twenty days later that three obese larvae developed. These transformed into soldiers. Emery was convinced that these results offer proof that food somehow controls castes, but the mechanism other than re-gurgitated feeding by the worker nurses he does not attempt to state. The study presents only a meager amount of data, and the conditions in the colony prior to manipulation alone serve as a control on the results. In addition, he failed to insure that the eggs or larvae from which the queens and soldiers matured were in

no way subjected to influences of the adults before those castes were removed.

A recent paper by Goetsch ('37) adds further evidence concerning effects of food on caste development. Eggs from very young (recently de-laid) queens were introduced into colonies where normal sized workers were raised. The workers from these eggs were again *small*, and only occasionally a little larger than those reared by a queen alone. They had developed according to the place from which they had come rather than according to conditions in their new environment. Goetsch postulates blastogenic determination in this case for smallness. There is a strong possibility, however, that the eggs of young queens are not as well nourished as later ones since they are produced by an insect that is no longer fed by a large population of workers as is an older queen in a flourishing colony. The fact that an occasional larger worker did develop in itself shows that such could happen and therefore smallness must not be genetically fixed.

Feeding experiments were made in which the populations were divided into halves and one group fed sugar or honey while the other had insect meat (flies). Soldiers were produced only when the larvae received a diet rich in meat. Older or better fed colonies would be expected to produce more soldiers, even if this caste were blastogenic, since a certain minimal environmental stimulus (food perhaps) is required to bring any set of genes to external expression. Thus Goetsch has not demonstrated that food alone is responsible, and further there was imperfect control over the castes present in the two colonies and their effect on the development of the larvae.

Occasional individuals intermediate between workers and soldiers were found, and it was thought these could be explained by the amount of food available to the ants. On the basis of this and other observations Goetsch asserts that transitional forms of *Pheidole* have not died out but are merely not produced

owing to certain external conditions affecting their development. Wheeler's records ('02) support Goetsch with respect to a correlation of increasing food supply with increasing morphological variability and distinctness. In my experiments a few soldiers have appeared which were dwarfed but were nevertheless true soldiers in relative proportions of their parts so they could hardly be classed as intermediates like the castes of *Atta* or *Pogonomyrmex*. It would seem more logical to suppose that most *Pheidole* have undergone a phylogenetic loss of the intermediate castes, and that this is due to a modification of genes (complex of species characters) which would otherwise permit intergrades. Either a complete soldier or none at all develops depending on the nutrition available. This does not indicate blastogenesis because the characters of castes like those of species do have representation in the germ plasm even though they may not depend upon separate genetic factors for differentiation. The soldier of one species is quite distinct genetically from that of another species, and this is attested by the fact that the soldiers are needed in many cases to separate species taxonomically. In regard to Goetsch's main conclusion, that the soldier is trophogenically determined, the facts seem to point in this direction, but whether such determination is based on food quantity alone is seriously questioned and cannot be decided from his data.

Wesson ('40) describes an experiment in which he fed one group of *Leptothorax curvispinosus* larvae on a superabundance of rich food and another sufficient food only to permit growth. A significantly greater proportion of queens developed in the better fed colony than in the poorer fed one. In addition he found that over-feeding during the summer months did not produce queens, but that after a five-month period of hibernation at 2° C. there seemed to be a marked tendency of some of the larvae to transform into queens. Perhaps he has discovered one of the mechanisms, aside from nutritional

factors, which differentiate workers from queens.

Castle's work ('34a, '34b) on *Zootermopsis* tends to substantiate Pickens's theory ('32) of inhibitory exudates. It was discovered that each caste prevents or delays the production of more individuals of the same type if it is present in sufficient numbers. Removal of a caste from an experimental colony allows one or more nymphs to mature into that caste. Thus kings, queens and soldiers are inhibited only by other members of their own caste, and the inhibitory substances conveyed by their secretions would appear to be caste-specific. These substances may be called "social hormones"; they are probably distributed by means of the trophallactic responses of the termites. Their effects were demonstrated through the use of extracts of the insect body. Filter paper impregnated with alcohol and ether solutions of functional queens was dried and fed to immature nymphs, resulting in a material increase of the time required for their complete development into supplementaries.²

EXPERIMENTAL

Procedure

The experimental approach to the study of caste origin in ants seems to present more difficulties than is the case with termites. All castes of the latter, including reproductives, can be raised in the laboratory, and their food is fairly uniform (cellulose). It is difficult to rear ant queens, and the food of ants is quite variable except that of the fungus-growers.

The dimorphic genus *Pheidole* was chosen for experimental material because its castes are very distinct and it is the most suitable ant of this type in the Chicago Region. *Pheidole morrisi* was discovered in sufficient numbers only in the dunes of the Kankakee River basin, ten miles south of Momence, Illinois. Two other species of *Pheidole* (*P. pilifera* and

² Dr. S. F. Light, University of California, regards the evidence as not entirely conclusive.

P. bicarinata) are obtainable in the area, but their colonies are too small or too infrequent to be practicable. It is necessary to gather colonies with queens because as yet it has been impossible to rear winged males and females. Moreover, the chances of their mating in captivity are uncertain. It is hoped that a technique for artificial insemination similar to that for bees (Watson, '27, '29) may be developed. Several advantages which *Pheidole* does possess, however, are its small size, adaptability to laboratory conditions and the distinctness of the castes at pupation. The manifestation of castes at a definite time, unlike the gradual nymphal changes of termites, makes it easy to form the correct diagnosis in an immature period. *Pheidole* pupae, fortunately, are not enclosed in cocoons. The greatest advantage is that the soldiers are independent of the workers in caring for themselves, the brood and the queen, and this permitted the study of brood development in pure soldier colonies.

The commonest nesting sites were in the bases of grass hummocks which grow in the small blowouts of the dunes. The colony personnel was collected into cloth sacks after having been dug quickly with a spade. The queen or queens (as many as four have been taken in one nest) when recovered were placed in a clean vial with a little moist sand to insure safe transportation. At the laboratory the sacks were emptied into separate "Forel arenas" and the sand allowed to dry. Dessication forces the ants to forsake their soil and take refuge in the moist dark chamber of a previously prepared plaster nest modified from the Janet-Wheeler type. The queen may then be introduced. Most nests had dimensions of 20×25 cm. with 1 cm. depth, but a few were $20 \times 12 \times 1$ cm., or $\frac{1}{2}$ the size (fig. 1). A median partition divided the nest into two chambers each $20 \times 12\frac{1}{2}$ cm., but left them connected by a narrow gap at one end. Water was supplied by means of a right-angled pipette secured into the outside wall and leading into a slice of silk sponge.

The sponge was supported on a thin glass plate to protect the shellacked plaster from deterioration. Clover honey and water (1 drop of distilled water to 1 drop of honey), and stripped mealworms were provided on separate glass squares which could be removed and cleaned. The honey was present in superabundant quantities at all times, and insect meat was introduced once or twice a week whenever counts were made. Each colony was given identical treatment at each manipulation for feeding and counting, so that any variations in regularity which crept in were experienced by all alike. Experiments 1-9 were carried out at room temperature, but the remainder were performed with the aid of an incubator. Temperatures in the incubator averaged from $26-27.5^{\circ}$ C., but occasionally reached 28° and once 29° . The lower limit of 25° was hardly ever attained as far as one could tell without a constantly recording thermograph. High relative humidity in the incubator was maintained with pans of saturated sand, but its range as measured by a hair hygrometer was quite wide. It varied usually from 60-75 per cent, but occasionally had extremes of 47, 53 and 80 per cent. The function of a fair degree of humidity in the incubator proper was to prevent rapid drying of the sponges inside the plaster nests. The latter were rather tightly covered with glass plates supported on pads of huck towelling, so that it may be estimated they maintained a relative humidity of 90 per cent or more although no device was available for measuring it. The ants did not "suffer" from minimal quantities of moisture however, as may be surmised from the hundreds of larvae that matured successfully.

Shifting of brood from one nest to another was accompanied by undelayed acceptance of the newcomers, and this was true to a considerable extent of adult ants transferred from an old to a new environment. Queens were accepted, usually, by queenless groups of workers in the few cases when this was necessitated by loss

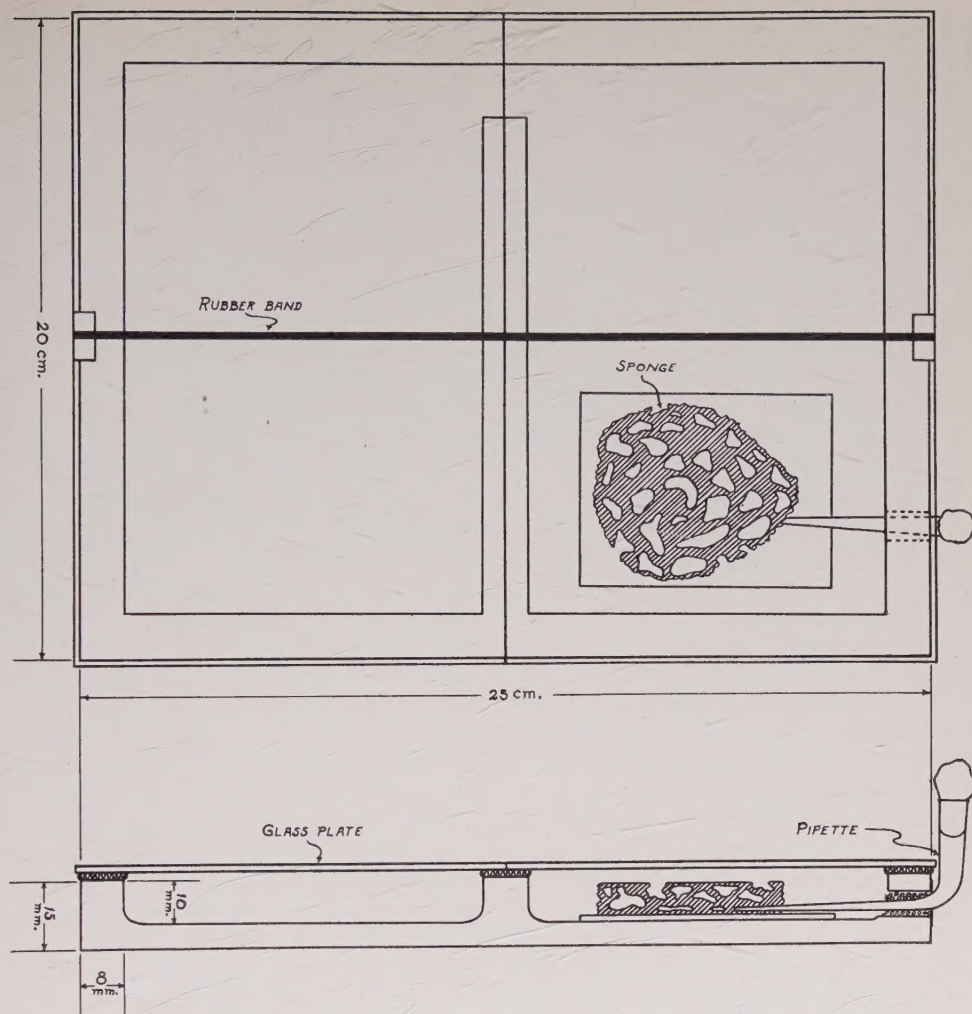


FIG. 1. Plan and cross-section of plaster nest used in the rearing experiments.

of worker personnel. It appeared that transfers were more successful if made while the ants were under the influence of ether and were then in a passive condition like the larvae. Whether this is the cause, or whether the ether removed some of the specific odors to which ants are extremely sensitive is not clear.

The purpose of these tests was to discover the rôle of the castes themselves in the determination of workers versus soldiers in contrast to the rôle of food as such. The investigations were stimulated by Castle's success, but were begun before

the present author was aware of the papers by Emery, Spencer, Wesson and Goetsch. They could not be patterned after Castle's experiments because of several differences in the habits of ants as compared with termites. At least one queen was needed per colony as a source of eggs. A transfer of over 200 eggs was made from a stock colony into a small queenless colony of about fifty workers. Only one individual reached the worker pupal stage; the rest were eaten or otherwise disappeared though large amounts of honey were provided. The rearing of

incipient colonies from seven different queens all from small nests collected in the Fall of 1937, failed completely, and even one that was begun with five workers and two soldiers succumbed. These results made it impractical to obtain extensive data by such methods, and it was necessary to resort to the keeping of large, well established colonies direct from the field, observing the outcome of brood development in them under varying conditions. With encouraging results from these experiments it may be possible eventually to devise a technique for rearing *Pheidole* in small containers under more perfectly controlled conditions.

After the nests were established and the ants had recovered from disturbance (few days to a week) they were etherized with high grade medical ether which gives a low mortality. The queens were removed immediately to protect them from the drug, and then the soldiers were picked out with fine watch-maker's forceps, counted and placed in a fresh nest. Soldiers from two or more colonies were combined while anaesthetized in order to make as large and strong a beginning as possible. This was necessary because soldiers though more tenacious than the workers, develop slower and are fewer in normal wild colonies. Thus artificial groupings of pure worker, and pure soldier colonies were produced. Controls consisted of colonies with both castes present just as they arrived from the field. When the numbers of one or the other caste became depleted (usually the soldier), more were added from other nests to maintain proportions as nearly constant as feasible with the material at hand. Soldiers developing in a worker colony and vice versa were removed periodically to keep the conditions near to those at the start (100 per cent one caste), but this could not be done frequently as etherizing tends to delay growth and disorganize the ants. Development took weeks even at the high temperature used.

It would have been desirable to prepare colonies with varying percentages of

castes between 0 per cent and 100 per cent to discover the threshold effect of a caste on differentiation. Again, this was not practical at the time but may be realized at a future date. The three alternatives described above gave a wide spread of conditions, and it was felt that if positive results were not produced by this technique no amount of percentage refinement would help. One of the chief difficulties sustained was the method of counting. Observations were made once a week and for populous colonies an automatic counter was used. In order not to excite the ants unduly, the cover for the darkened chamber was replaced with a plate of amber glass. Enough light was admitted if a powerful spot lamp supplied the illumination, yet the blue end of the spectrum to which ants are very sensitive was excluded and their normal activities were not interrupted. The warmth agitated them sufficiently to cause removal of the brood from one spot to another and this dispersal of the packets facilitated counting. Toward the end of the experiments a type of nest was constructed in which no sponge was used since moisture was allowed to seep through the porous plaster from a watering depression in the side. Counting was made easier and more accurate by this method, but some trouble was experienced from mould. A low power of the dissecting microscope was employed regularly to insure that each individual was seen clearly and its caste diagnosed correctly. Counts were repeated until it seemed certain the error was small. The accuracy of the figures leaves room for improvement, but the high numbers involved militated against any serious defect from slight mistakes. The soldier pupae were so distinct and so few relative to the workers that it is felt their counts are reliable. Then too, a check was afforded by the number which emerged into adults, and a final check was obtained by very accurate counts of the ants at the start and at the finish of an experiment. The dead were tallied also and added to those left alive so that the

number of ants which passed through a colony during the period of observation was known. Weekly counts were made in an effort to include individuals that developed partially but which were subjected to cannibalism. In the death pile the bodies were nearly always dismembered, but the head capsules remained intact which permitted an accurate count of individuals. Mould, mites and booklice sometimes invade a colony, but are fairly well controlled by the ants. They seem to chase away the booklice, but mites are more troublesome and probably more damaging to the brood because they multiply rapidly. It is doubtful, however, if these parasites and predators had any detrimental effects on the experiments since the hundreds of larvae which reached adulthood probably had not been attacked. The workers assiduously cared for the young protecting them from all predation except their own cannibalism. There are undoubtedly some scavengers on the refuse piles of wild nests, and in captive colonies they appear to stay chiefly on the "kitchen middens."

It was extremely fortunate that ants of both castes could be brought to maturity

in pure soldier colonies. Soldier *Pheidole* were observed to lick and feed the larvae and delicately carry the eggs despite their powerful jaws. The queen was seen being groomed and fed, and in all respects the colony behaved as normally as if workers had been present. This is further substantiated by the fact that hundreds of workers and some soldiers developed properly under these conditions.

RESULTS

The outcome of thirty-three experiments is summarized in tables I, II and III. For example, in table I the following is an account of the events in experimental colony Number 8. There were 39 workers and no soldiers present when the colony was started, representing respectively 100 per cent and 0 per cent of these castes (a pure worker colony). When the experiment was terminated, 18 workers and 2 soldiers were present, or a percentage composition of 90 per cent workers and 10 per cent soldiers. During the period of observation (4.6 months on the average for each colony) 166 workers and 4 soldiers developed. These figures were computed from the raw data by

TABLE I. *Worker Experiments*

Exp. No.	Adults Present				Adult %				Brood Developed		Brood %		Brood Index	Brood Ratio	No. of Workers at 1st Soldier	No. of Soldiers at 1st Soldier
	Start		Finish		Start		Finish		W	S	W	S	W to S	W to S		
	W	S	W	S	W	S	W	S								
1	50	0	66	9	100	0	88	12	140	9	93.9	6.1	0.064	15.5:1	?	?
3	54	0	150	3	100	0	98	2	114	3	97.4	2.6	0.026	38:1	?	?
4	163	0	333	13	100	0	96.2	3.8	170	13	92.9	7.1	0.076	13:1	?	?
5	906	0	332	17	100	0	95	5	125	17	88.0	12.0	0.136	7.3:1	?	?
8	39	0	18	2	100	0	90	10	166	4	97.6	2.4	0.024	41.5:1	45	0
10	60	0	41	1	100	0	97.6	2.4	176	3	98.3	1.7	0.039	58.6:1	56	0
11	170	1	73	13	99.4	0.6	84.8	15.2	207	33	87.3	12.7	0.159	6.27:1	170	1
12	125	1	97	7	99.2	0.8	93.2	6.8	115	7	94.2	5.8	0.061	16.4:1	125	1
15	66	0	1	0	100	0	100	0	4	0	100.0	0.0	0.000	4:0	—	—
16	84	0	235	5	100	0	97.9	2.1	508	8	98.4	1.6	0.016	63.4:1	76	0
17	50	0	99	1	100	0	99.0	1.0	279	8	97.2	2.8	0.028	34.8:1	115	0
25	2,233	0	308	40	100	0	88.5	11.5	172	149	53.5	46.5	0.866	1.15:1	1,678	0
29	2,008	0	655	125	100	0	84.0	16.0	947	188	83.4	16.6	0.198	5.03:1	1,665	0
31	3,580	0	1,703	23	100	0	98.6	1.4	101	23	81.4	18.6	0.227	4.39:1	2,387	0
32	12	0	88	3	100	0	95.6	4.4	260	10	96.2	3.8	0.038	26:1	63	0
33	20	0	1	0	100	0	100	0	4	0	100.0	0.0	0.000	4:0	—	—
34	60	0	161	9	100	0	94.7	5.3	941	11	98.8	1.2	0.011	85.5:1	140	0
Av. of 17	569	0.12	257	16	99.9	.008	94	6	261	29	91.6	8.5	0.115	25:1	593	0.36
Total									4,429	486						

Standard Error of averaged indices (0.115) = 0.04939.

TABLE II. *Soldier experiments*

Exp. No.	Adults Present				Adult %				Brood Developed		Brood %		Brood Index	Brood Ratio	No. of Workers at 1st Soldier	No. of Soldiers at 1st Soldier	
	Start		Finish		Start		Finish		W	S	W	S	W to S	W to S			
	W	S	W	S	W	S	W	S									
6	0	107	41	52	0	100	53	47	41	0	100	0	0.000	41:0	—	—	
7	0	27	2	2	0	100	50	50	2	0	100	0	0.000	2:0	—	—	
13	3	36	24	3	11	92	89	8	146	1	99	1	0.0068	146:1	105	26	
14	0	28	32	16	0	100	66.7	33.3	41	0	100	0	0.000	41:0	—	—	
18	0	115	217	26	0	100	89.3	10.7	247	1	99.6	0.4	0.004	247:1	1	11	
19	0	18	1	7	0	100	12.5	87.5	2	0	100	0	0.000	2:0	—	—	
23	0	222	620	25	0	100	96	4	742	6	99	1	0.008	123.6:1	56	142	
24	0	300	509	106	0	100	82.7	17.3	693	3	99.5	0.5	0.004	231:1	173	244	
27	0	360	454	10	0	100	97.8	2.2	549	5	99	1	0.009	109.8:1	178	151	
30	0	327	50	17	0	100	74.6	25.4	347	2	99.4	0.6	0.006	173.5:1	60	105	
Av. of 10	0.3	154	195	26.4	1.1	99.2	71.3	28.5	281	1.8	99.6	0.5	0.0038	112:1	96	113	
Total									2810	18							

Standard Error of averaged indices (0.0038) = 0.00113.

TABLE III. *Controls*

Exp. No.	Adults Present				Adult %				Brood Developed		Brood %		Brood Index	Brood Ratio	No. of Workers at 1st Soldier	No. of Soldiers at 1st Soldier	
	Start		Finish		Start		Finish		W	S	W	S	W to S	W to S			
	W	S	W	S	W	S	W	S									
2	23	14	12	3	62	38	80	20	92	7	93	7	0.076	13.14:1	?	?	
9	55	17	47	13	76.3	23.7	78.3	21.7	51	10	83.6	16.4	0.196	5.1:1	?	?	
20	50	1	5	0	98	2	100	0	86	0	100	0	0.000	86:0	—	—	
21	48	15	177	7	76	24	96.2	3.8	384	8	98	2	0.028	48:1	45	3	
26	111	90	319	12	55.2	44.8	96.4	3.6	643	136	82.5	17.5	0.211	4.7:1	188	39	
28	687	231	312	158	74.8	25.2	66.4	33.6	408	5	98.7	1.3	0.012	81.6:1	312	151	
Av. of 6	162	61	145	32	73.6	26.3	86	14	277	27	92.8	7.3	0.087	39.8:1	181	64	
Total									1628	166							

Standard Error of averaged indices (0.087) = 0.0383.

adding the number of individuals of each caste remaining alive (18 W—2 S) to those which had died during the course of the experiment (187 W—2 S), making a total of 205 workers to 4 soldiers involved in the whole colony, and then subtracting the numbers of adult castes present at the start (205 minus 39 equals 166 workers; and 4 minus 0 equals 4 soldiers). The percentages of workers and soldiers which thus developed were 97.6 per cent and 2.4 per cent respectively.

The index between soldiers and workers was calculated and found to be 0.024. Similarly, the ratio (the reciprocal) between these two castes in the brood was found to be 41.5 to 1. The number of workers present when the first soldier appeared had risen from 39 to 45. Statistical tests show significant differences between the numbers of soldiers produced in worker colonies as compared with those in soldier colonies or control colonies. Table IV shows the results of

TABLE IV. *Significance of indices*

Counts	Worker vs. Soldier		Soldier vs. Control	Worker vs. Control
Pupae	Computed Totals	0.0000	0.1615	0.0009
	Actual Totals	0.0000	0.1096	0.0000
Adults		0.0357	0.0891	0.3910
Mixture		0.025	0.044	0.66

these calculations. Instead of comparing numbers directly, owing to variability amongst figures, an index was taken between workers and soldiers that developed in each colony. Presumably this index should be different depending on whether the results are from a worker, a soldier or a control colony. The indices were averaged for both types of experimentals and for the controls. Worker versus soldier, soldier versus control and worker versus control comparisons were made. The indices from pupal counts, from adult counts and from mixtures of these data were tested separately. Only the last group (mixed data) is included in the tables of data as it is considered most reliable. The pupal indices were derived from actual numerical totals of the pupae present on successive counts, the "actual totals," and from probable figures which take into account the overlap from one clutch of eggs to another, the "computed totals." In all cases the "probability" values for worker versus soldier colonies were less than 0.05 indicating a significant difference in the production of soldiers in the two types. The differences between the indices of soldier as compared with control colonies and worker as compared with control colonies are on the whole insignificant and less uniform than the differences between the indices of worker as compared with soldier colonies. This may be due to inadequacies inherent in the methods of collecting data or to a possible threshold effect. In the latter instance it might be that the proportions of workers and soldiers in the controls were not suffi-

ciently different from those in either pure worker or pure soldier experiments to show a distinct response in brood development. The worker and soldier experiments themselves are at polar extremes, and this may account for their display of marked differences in the brood products under the two environments.

From the figures in tables I, II and III it will be seen that the development of soldiers tends to be prevented in soldier colonies especially, but that in worker or control colonies there is decidedly less interference with their growth. In this connection it is desirable to call attention to the following facts. The average brood ratio between workers and soldiers for the worker experiments was 25 to 1 respectively, for the soldier experiments 112 to 1 and for the controls 39.8 to 1. There would seem to be a definite reduction in the development of soldiers in the soldier experiments, and a lessening of this effect in the worker experiments as indicated by the intermediate position of the ratio for the controls. The averaged indices show essentially the same phenomenon: for the worker experiments the indices averaged 0.115, for the soldiers 0.0038 and for the controls 0.087.

The ratios between castes in normal, wild colonies of *Pheidole morrisi*, *P. bicarinata* and *P. pilifera* are given in tables V, VI and VII respectively. These may be used as normal datum planes with which to make comparisons of the ratios of the experiments. For example, the average ratio between castes for the three species is 7-9 workers to 1 soldier in the adult population, but the average ratio between developing castes of the worker experiments is 25 to 1 as compared with 112 to 1 for the soldier experiments. While the average for a colony in the field as indicated above is about 8 to 1, individual nests show wide variations. It is interesting to note also that the three species are in close agreement for the average ratio. The discrepancies among the various counts are to be interpreted in part as a function of the age and vigor of the

TABLE V. *Normal caste counts of Pheidole morrisi* Forel

Col. No.	Counts		Percentage		Index W to S	Ratio W to S	No. of Queens
	W	S	W	S			
1	26	1	97.3	2.7	0.04	26:1	0
2	193	18	91.5	8.5	0.093	10.7:1	0
3	58	0	100	0	0.000	58:0	0
4	407	55	84.6	15.4	0.135	7.4:1	0
5	32	5	86.5	13.5	0.156	6.4:1	0
6	23	12	65.7	34.3	0.520	2:1	0
7	8	2	80.0	20.0	0.250	4:1	2
8	156	72	68.4	31.6	0.461	2.16:1	0
9	585	124	82.5	17.5	0.212	4.72:1	0
10	464	47	90.8	9.2	0.101	9.87:1	12 winged
11	192	27	87.7	12.3	0.140	7.1:1	1
12	2,233	522	81.0	19.0	0.234	4.27:1	3
13	2,679	386	87.4	12.6	0.144	6.94:1	3
14	166	108	60.6	39.4	0.650	1.53:1	1
15	301	163	64.9	35.1	0.541	1.84:1	1
16	577	125	82.2	17.8	0.216	4.62:1	0
Av.	506.25	104.20	81.9	18.1	0.243	9.8:1	1.44

Standard Error of averaged indices (0.243) = 0.0485.

TABLE VI. *Normal caste counts of Pheidole bicarinata* Mayr

Col. No.	Counts		Percentage		Index W to S	Ratio W to S	No. of Queens
	W	S	W	S			
1	80	8	90.9	9.1	0.100	10:1	0
2	21	1	95.4	4.6	0.048	21:1	0
3	7	1	87.5	12.5	0.143	7:1	0
4	56	11	84.8	15.2	0.196	5.09:1	4 winged, 2 ♂
5	26	2	92.8	7.2	0.077	13:1	0
6	408	24	94.4	5.6	0.059	17:1	0
7	7	1	87.5	12.5	0.143	7:1	0
8	7	0	100	0	0.000	7:0	0
9	6	0	100	0	0.000	6:0	0
10	12	0	100	0	0.000	12:0	0
11	6	0	100	0	0.000	6:0	0
12	53	18	74.6	25.4	0.340	2.9:1	0
13	24	4	85.7	14.3	0.167	6:1	0
14	45	3	93.7	6.3	0.066	15:1	0
15	33	22	60	40	0.670	1.5:1	0
16	61	2	96.8	3.2	0.033	30.5:1	0
17	71	11	86.5	13.5	0.154	6.45:1	0
18	23	3	88.4	11.6	0.130	7.66:1	0
19	59	5	92.2	7.8	0.085	11.8:1	0
20	11	3	78.5	21.5	0.273	3.66:1	0
21	42	3	93.3	6.7	0.071	14:1	0
22	15	0	100	0	0.000	15:0	0
23	17	5	77.2	22.8	0.294	3.4:1	0
24	22	2	91.6	8.4	0.090	11:1	0
25	28	2	93.3	6.7	0.071	14:1	1, 8 ♂
26	110	26	80.8	19.2	0.236	4.2:1	0
27	259	18	93.5	6.5	0.069	14.3:1	0
28	23	5	82.1	17.9	0.217	4.6:1	0
29	89	14	86.4	13.6	0.160	6.36:1	5 pupae
Av.	56	6.7	89.24	10.76	0.134	9.77:1	1

Standard Error of averaged indices (0.134) = 0.0257.

colonies and in part to errors in sampling although efforts were made to obtain as much of the population of a nest as possible in each case. Figure 2 shows graphic comparisons of the results of the experi-

TABLE VII. *Normal caste counts of Pheidole pilifera* (Roger)

Col. No.	Counts		Percentage		Index W to S	Ratio W to S	No. of Queens
	W	S	W	S			
1	10	1	90.9	9.1	0.100	10:1	0
2	43	7	86	14	0.162	6:1	0
3	9	0	100	0	0.000	9:0	0
4	43	9	82.7	17.3	0.209	3.8:1	0
5	69	17	80.2	19.8	0.246	4:1	0
6	41	7	85.4	14.6	0.170	6:1	0
7	67	6	91.8	8.2	0.089	11:1	0
8	60	6	90.9	9.1	0.100	10:1	1
Av.	42.75	6.6	88.5	11.5	0.135	7.54:1	1

Standard Error of averaged indices (0.135) = 0.02755.

ments and of the normal caste counts for three species, and though the standard errors are large, there is no overlap between the soldier colonies on the one hand, and workers and controls on the other.

DISCUSSION

A. Points of weakness.—The reported experiments merely make a beginning in the analysis of caste problems in ants, and some of the criticisms which may be made follow.

1. Although several thousand ants (9,537) were observed and their determination noted, the numbers to develop in any one experiment were rather low; the highest were 947 workers to 188 soldiers and the lowest 2 workers to no soldiers. Colonies 15 and 19 for example comprise such small numbers that they are of little significance. The number of experiments of each class should have been higher, but was limited by the availability of material and difficulties incident to its collection such as the scarcity of queens. In all there were 17 worker experiments, 10 soldier experiments and 6 controls. The number of the latter seem partially justified in view of the data on normal caste counts. From these it can be seen that the proportions of castes in nature vary enormously; therefore it is rather difficult to decide what the percentages should be in a control nest. It is of interest to ob-

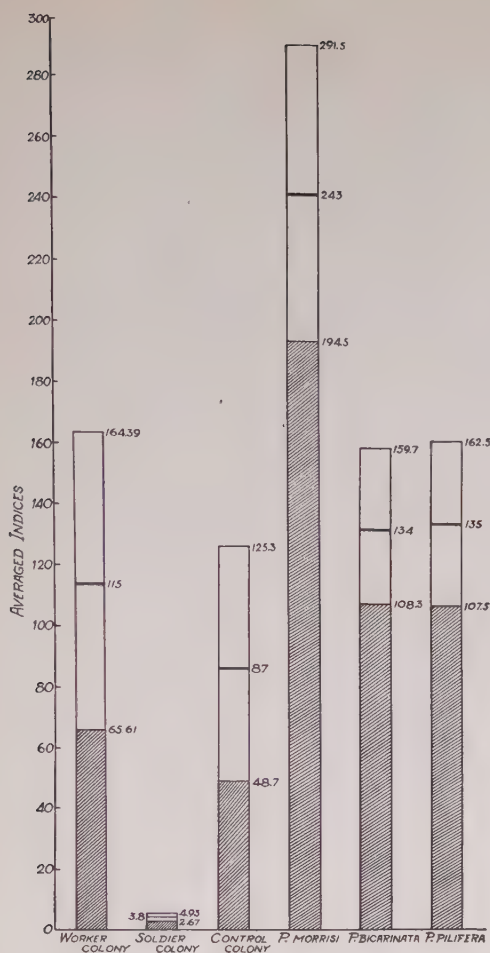


FIG. 2. Graphic comparisons of the significance of indices. The indices of worker, soldier and control colonies of laboratory populations of *Pheidole morrisi* (averaged) are shown with their respective standard errors (in white). Indices for wild colonies of three species of *Pheidole* (*P. morrisi*, *P. bicarinata* and *P. pilifera*) are included for purposes of further control.

serve that the percentages of workers and soldiers changed less in the controls during the course of the tests than in the soldier experiments.

2. The control over possible hereditary factors was not complete in that the experiments were concluded before realizing the advisability of shifting a given queen from a pure worker to a pure soldier colony and vice versa. Observa-

tions on the development of eggs from the same individual under differing conditions would go far toward proving or disproving the genetic identity of eggs.

3. It was not practical to keep experimental colonies of the same size (numbers) because of the paucity of ants for stock cultures from which to regulate excesses and deficiencies. Most tests were made in nests of equal size. The volume of nest space above a minimum is regarded as unimportant within moderate range because social animals normally crowd themselves even when much space is available. Crowding could limit the total size of the population, but if enough food were present it seems extremely unlikely that this factor would loom large in determining an adaptive caste like *Pheidole* soldiers. Undercrowding in too large a nest chamber might cause harmful dilution of the ants' energy for foraging and mould control beyond the point where they could raise soldiers or any brood at all.

4. While food was kept constant in quality, it was not absolutely constant in quantity. This is of no consequence, however, because an excess of diluted honey was supplied at all times and the insects ate whenever food was required. When mealworms were given, each colony was supplied in excess. They were fed in the same manner and at the same time.

5. The difficulties of accurate counting, as described above, were overcome by comparing indices rather than actual figures. Frequent use of ether for the purpose of making accurate counts would require at least an hour to complete the tally in populous colonies and is ruled out owing to the disturbance it produces. In one instance a soldier experiment (Number 30) failed to rear any further brood after being heavily etherized to remove the developed workers and the result seems to have been correlated with the anaesthetic.³

³ Success in the use of carbon dioxide on *Camponotus* has been reported by Mr. Falconer Smith in a personal communication.

B. Conclusions.—

1. Experimental modifiability of brood caste percentages.—The results indicate that the development of a given larva can be profoundly changed by the percentage composition of the adult castes in a colony. The much greater number of soldier pupae and adults appearing in a nest composed only of workers than in a soldier nest, where the conditions both biotic and physical are kept essentially constant in quality, shows that the population as such cannot be neglected in an interpretation of the origin of polymorphism. The colony behaves as a unit and automatically approaches an equilibrium by adjusting the percentages of the castes if they become shifted from the "normal" condition for the species. The experiments represent extreme examples of these shifts.

2. Soldier caste controlled by the number of soldiers present with the possibility of a soldier inhibiting hormone also.—Where this caste is present in large numbers (100 per cent), there is a decrease in the number of soldiers that are produced. Conversely, in pure colonies of workers an excess of soldiers tends to appear over the number which normally develop in a control colony. A social hormone similar to that postulated for termites is considered probable since there seems to be an inhibition of soldier development. The substance could be readily transferred through a certain number of workers in control colonies or directly from the soldiers in a soldier nest by means of the observed trophallactic relations of all members. Supposedly, it would be conveyed in exudates of the insects' bodies, and would gain entrance into the general trophic circulation through the grooming habits of the ants.

3. Quantitative food control unlikely.—Since food quantity in these experiments was sufficient for the growth of many soldiers and an even larger number of workers, and since soldiers develop in rather small colonies both in the laboratory and in nature, it is improbable that quantitative feeding could be responsible for the ob-

served differences. The quantity of food was approximately the same in each colony (see A, 4 above). Premature pupation, as suggested by Huxley ('27), might account for the production of the diminutive caste, or worker (supposedly due to insufficient food), and full growth before pupation might account for the production of soldiers. If this is true, soldiers would be expected to appear in considerable numbers in well fed soldier colonies but such was not the case. The production of soldiers in a soldier nest did not materialize until a considerable period *after* the appearance of workers. A few workers could supplement the efforts of the soldiers if the latter were inefficient as nurses, but the development of soldiers was delayed until the worker population had accumulated to rather high numbers (table II). Again, the results point to an inhibition by the soldier adults which is not relieved until sufficiently diluted by a large number of workers.

4. Origin of worker caste unsolved.—The fact that worker ants were produced in large numbers in all colonies, and were about as numerous in worker as in soldier nests indicates that the presence of the adults has little if any inhibitory effect on the development of more workers.

5. Genetic differences in the eggs in the sense of simple caste determining genes is refuted.—It seems to be adequately demonstrated that the eggs of *Pheidole* are identical with respect to caste potentialities, and that the soldiers at least are trophogenically determined. The late and rather sudden assumption of definite caste characters allows the possibility (in the light of other supporting data) that caste determination does not occur before hatching. The evidence appears to indicate lack of fixation until some comparatively late larval stadium.

C. *Theoretical considerations.*—The bearing of these experiments on the various facts and opinions concerning polymorphism is evident, for if the results are valid they support and extend the trophogenic hypothesis. Observations on mer-

mithergates, pseudogynes and intercastes (Wheeler, '28) show some disturbance in the nutritive influences on caste origin. Even the striking picture presented by gynergates and gynandromorphs can be resolved probably on the basis of thresholds.

It may still be thought that intrinsic origin has not been successfully refuted. In a recent paper Goetsch ('39) states that an intermediate nasute type soldier appeared in a captive colony of *Anoplotermes* (soldierless genus), and can be interpreted as furnishing evidence for genetic differences in the eggs. It would mean that although soldiers do not appear in normal wild colonies and are supposed to have dropped out phylogenetically, their genes are in reality still retained by the species. This can be interpreted in favor of environmental control by assuming that, while genes are admittedly present, some change in the physiological thresholds in the particular colony has in part enabled them to come to expression. The anomaly might also be interpreted as the result of phylogenetically suppressed genes which cause the degeneration of certain characters (in this case the soldier caste), but which have not been entirely lost because of other vital influences in the termite colony. Finally, Hare ('37) shows that *Anoplotermes* is not closely related to the nasute termites (Nasutitermitinae) so that the discovery of a nasute soldier in this genus seems inconsistent and needs further verification.

There is no doubt that caste characters are genetic because these traits are genus and species specific. This is quite different, however, from supposing special determiners (Weismann, '93) which delegate whole eggs to one caste or another beyond the possibility of redifferentiation. It would seem that each egg contains a complete set of all genes belonging to the species which necessarily includes those of all polymorphic characters (Emerson, '39). During development these genes interact with internal and external environmental

stimuli and inhibitors in such a way that only those genes responsible for the traits of one caste are allowed to come to morphological, physiological and behavioristic expression. To which set of environmental stimuli a given egg or larva will be subjected is problematical and may be experimentally investigated. The individual ants are analogous to the cells in their bodies. Each has all the potentialities of its species, and those potentialities that develop depend on the conditions to which those individuals and cells are subjected.

SUMMARY

1. Experiments were performed on *Pheidole morrisi* in which the ants were reared from egg to adult in colonies composed only of workers, others only of soldiers and controls consisting of both workers and soldiers.

2. A significantly greater proportion of soldiers developed in the worker colonies than in either the soldier or control colonies. Virtual inhibition of the caste was obtained in soldier colonies.

3. The workers appeared to develop about equally well in all three colony types.

4. A special substance contained in an exudate of the soldier body and distributed to the larvae by the grooming habits of the nurses is postulated for the inhibition of the soldier caste.

5. Soldiers are extrinsically determined, probably after the hatching of the eggs. Worker determination is as yet unknown.

6. The results of this study are in accord with a preponderance of the observational data.

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